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STUDIES ON ANTIBACTERIAL ACTIVITY OF *Adenium obesum* (*Apocynaceae*) STEM-BARK

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ABSTRACT
Antibacterial evaluation of the methanolic and petroleum spirit (60-80) ^{o}C extracts of the stem-bark of *adenium obesum* was carried out using agar- well diffusion method. The extracts were tested against some selected standard gram negative bacteria strains–*Escherichia coli (*ATCC 25922), *Pseudomonas aeruginosa (*ATCC- 25619), *Salmonella typhi(*ATCC 9184), *Neisseria gonorrhea* (NCTC 8198), *Klebsiella pneumonia*(ATCC 15380). Antimicrobial sensitivity test of the extracts at concentration of 4×10^4 $\mu g/cm^3$ showed significant zones of inhibition against 80% of the tested organisms. The minimum inhibitory concentration (MIC) of the extracts against the listed organisms ranges from 1×10^4 $\mu g/cm^3$ to 4×10^4 $\mu g/cm^3$ and 3×10^4 $\mu g/cm^3$ to 5×10^4 $\mu g/cm^3$ for Crude extracts of the methanol and petroleum spirit respectively. Phytochemical examination of the extracts revealed the presence of alkaloids, steroids, saponins, glycosides, anthraquinones, tannins and flavonoids. These findings reveal broad spectrum activity of the plant stem-bark extracts.

KEYWORDS: Gram Negative, bacterial studies, Stem-bark, *Adenium obesum*

INTRODUCTION:

The African environment is probably the least explored in terms of available untapped resources. Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potentials of introducing new templates into modern medicine.

The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into African continent (Akinyemi *et al*, 2005). Herbal medicine has been widely used and formed an integral part of primary health care in China (Liu, 1987) Ethiopia (Desta, 1993) Argentina (Anesini and Perez, 1993) and Papau New Guinea (Nick *et al*, 1995). A significant proportion of pharmaceutical products in current use are designed from plants (Cowan, 1999 and Rankin *et al*, 2002). A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effect on all types of micro-organisms in vitro (Cowan, 1999) and some plant extracts have shown activity on both gram negative and gram positive organism (Nascimento *et al*,2000) .

The use of various plant parts in the treatment of the sick developed into tradition which was handed down from one generation to another over the years verbally or written (Sofowora, 1982; Akinyanju, 1986). For thousands of years, medicine depended exclusively on leaves, flowers and barks of plants, only recently have synthetic drugs come into use and in many instances, these are replicas of chemicals identified in plants (Conway, 1973). In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients is extracted refined and made ready for consumption, while in traditional medicine, a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food (Conway, 1973).

Even before the discovery of modern antibiotics and other chemotherapeutic agents, traditional medicine has served as man's resort when attacked by infective agents such as bacteria and fungi (Crafton, 1983).

The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on enthno-medicinal plants in Nigeria and Africa. Nevertheless, the increased incidence of diseases for which there is yet an effective remedy is a driving factor for more screening programmes. Diseases like tuberculosis, pneumonia, typhoid fever, rheumatic fever and meningitis

(Greenwood *et al*., 1992) still pose a major challenge to modern chemotherapeutic agents. It is in this context that the stem-bark of *adenium obesum* was screened for antibacterial activity.

Adenium obesum belongs to the family *Apocyanaceae*. It is a Succulent shrub or small tree, up to 4(–6) m tall, sometimes with a fleshy taproot; stem swollen at base up to 1(–2) m in diameter; bark pale greyish-green, grey or brown, smooth, with sticky, clear or white latex; branchlets glabrescent, pubescent at apex. Leaves arranged spirally, clustered at the end of branchlets, simple; stipules minute or absent; petiole up to 4 mm long; blade linear to obovate, 3–12(–17) cm × 0.2–6 cm, base cuneate, apex acute to rounded or emarginate, entire, slightly glaucous, dull green or pale green, leathery, pinnately veined with distinct or indistinct lateral veins (Rowley,1983).

The plant is important in traditional medicine. In the Sahel a decoction from the roots, alone or in combination with other plants, is used to treat venereal diseases; a root or bark extract is used as a bath or lotion to treat skin diseases and to kill lice, while latex is applied to decaying teeth and septic wounds. In Somalia a root decoction as nose drops is prescribed for rhinitis. In northern Kenya latex is rubbed on the head against lice and powdered stems are applied to kill skin parasites of camels and cattle. The bark is chewed as an abortifacient (Neuwinger, 2000).

MATERIALS AND METHODS

Collection and Preparation of Plant Material

The stem-bark of *Adenium obesum* was collected from Samara–Zaria, Kaduna State of Nigeria. It was confirmed and authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria where a voucher specimen was deposited. The bark was air dried and ground into powdered form using a porcelain mortar and pestle. It was then preserved well in polythene bag and stored in a dessicator before subsequent experiments.

Extraction

The powered stem-bark of *Adenium obesum* (500g) were packed into a thimble and placed inside a soxhlet extractor and extracted exhaustively and respectively with petroleum spirit (60-80) ^{o}C and methanol. The extracts were concentrated *in vacuo* at 40 ^{o}C using rotary evaporator, after which the crude extracts were obtained from the solvents.

Phytochemical screening

The petroleum and methanolic extracts of the stem-bark of *Adenium obesum* were subjected to

Preliminary phytochemical tests using standard techniques (Trease and Evans, 1989; Sofowora, 1993).

Test microorganisms

Five standard Gram-negative bacterial strains were selected; *Escherichia coli (*ATCC 25922), *Pseudomonas aeruginosa (*ATCC- 25619), *Salmonella typhi* (ATCC 9184), *Neisseria gonorrhea* (NCTC 8198) and *Klebsiella pneumonia* (ATCC 15380).The bacterial strains were maintained on nutrient agar and sub-cultured every three days. An inoculum of each bacterial strain was suspended in 5 ml of Mueller Hinton broth (MHB) and incubated overnight at 37 ºC. The overnight cultures were diluted with Mueller Hinton broth and adjusted to give a concentration of bacterial cells equivalent to a McFarland 0.5 standard prior to the bacterial testing (Samie *et al*., 2005).

Susceptibility testing

The agar cup diffusion method was used for this test. Sterile nutrient agar plates were flooded with appropriately diluted microorganism, the excess was aseptically drained and the surface, allowed to dry at room temperature. Wells were bored into the agar plates using a 4 mm sterile cork borer and 0.1 ml each of the extracts at concentration of $4\times10^{4}\ \mu g/cm^{3}$ was introduced into each well. The plates were allowed a pre-diffusion time of 1 h at room temperature and then incubated at 37°C for 24 h after which zones of inhibition were read to the nearest millimeter.

Minimum inhibitory concentration [MIC]

A series of culture tubes (microdilution assays) were prepared all containing the same volume of medium inoculated with test microorganisms (Ferreira *et al*, 2003). The lowest concentration of sample at which the subculture from test dilution yielded no viable organisms was recorded as minimum inhibitory concentration (Nazaruk and Jakoniuk, 2005). Decreasing concentration of petroleum spirit or methanol extracts of *Adenium obesum* (AO) extracts was added to the tubes usually a step wise dilution (two fold serial dilutions) was used starting from highest to lowest concentrations. One tube was left without AO extracts to serve as positive control and other without extract and inoculums to serve as negative control. The cultures were incubated at a temperature optimal for growth of the test organism and a period of time sufficient for growth for at least 10-15 generations (usually 24 hours for bacteria at 37 ° C). The tubes were inspected visually to determine the growth of microorganisms by the presence of turbidity and the tubes in which AO extract is present in minimum concentration sufficient to inhibit the microbial growth which remains clear was noted as MIC of the extract. In experimental terms MIC is the concentration of the AO extracts present in the last clear tube that is the tube having the lowest antibiotic concentration in which growth is not observed.

RESULTS AND DISCUSSION

Table 1: Phytochemical constituents of the stem-bark of *Adenium obesum*

Constituents	Extract	
	Petroleum spirit	Methanol
Alkaloids	_	+
Flavonoids	_	+
Saponins	+	+
Glycosides	+	+
Anthrquinones	_	+
Tannins	+	+
Steroids	+	+
Coumarins	_	_

+/_ = presence or absence of constituent tested

Table 2: Antibacterial sensitivity tests of the crude extracts of *Adenium obesum* (stem-bark*)* at Concentration of $4\times10^{4}\ \mu g/cm^{3}$: Zone of Inhibition (mm).

Test organisms	Zone of Inhibition (mm)	
	Methanolic extract	Petroleum spirit extract.
*Escherichia coli(*ATCC 25922)	26	20
*Pseudomonas aeruginosa (*ATCC- 25619)	-	-
*Salmonella typhi(*ATCC 9184)	20	16
Neisseria gonorrhea (NCTC 8198)	27	17
Klebsiella pneumonia(ATCC 15380)	22	22

⁻ = No zone of inhibition

Table 3. Minimum inhibitory concentrations (MICs) of the petroleum spirit and ethanol extracts of the stem-bark Adenium obesum

Microorganism	MIC ($\times 10^4 \mu g / cm^3$)	
	Petroleum spirit extract	Methanol extract
*Escherichia coli(*ATCC 25922)	4	2
*Pseudomonas aeruginosa (*ATCC- 25619)	NG	NG
*Salmonella typhi(*ATCC 9184)	5	3
Neisseria gonorrhea (NCTC 8198)	4	2
Klebsiella pneumonia(ATCC 15380)	3	3

NG = No Growth

Preliminary phytochemical screening: The preliminary phytochemical screening of the plant extracts revealed the presence of alkaloids, steroids, saponins, glycosides, anthraquinones, tannins and flavonoids in the methanol extract. However, coumarins were absent in the extract. While the petroleum spirit extract contained only saponins, glycosides, tannins and steroids; alkaloids, flavonoids, anthraquinones and coumarins were absent (Table 1). The methanolic crude extract contained more secondary metabolites than the petroleum spirit extract probably because of the polarity of the solvent (methanol).Methanol as more polar solvent is capable of extracting both the polar and the non-polar components of the plant stem-bark and hence having more metabolites in it.

Antimicrobial tests: The plant extracts have demonstrated significant antimicrobial activity against the microorganisms. The two extracts (methanol and petroleum spirit) are active against four out of the five organisms tested. The inhibition zones for methanolic extract, which ranges from 20-27mm, were generally higher than that of petroleum spirit; with range from 16-22mm (Table 2).This reveals greater antimicrobial activity of the methanolic extract compared with that of petroleum spirit.

MIC values of the methanolic extract were smaller (more active) than that of petroleum spirit. The smallest MIC ($2\times 10^4 \mu g / cm^3$) was obtained with *Escherichia* coli (ATCC 25922) and *Neisseria gonorrhea* (NCTC 8198). This is a good manifestation of antibacterial activity of AO extract. For the petroleum spirit extract the MIC values for the same microorganisms was $4x10^4 \mu g/cm^3$. The highest MIC value for the AO methanolic extract was $3x10^4 \mu g/cm^3$ against *Salmonella typhi* (ATCC 9184) and *Klebsiella pneumonia* (ATCC 15380), while that of petroleum spirit extract is $5x10^4 \mu g/cm^3$ against *Salmonella typhi* (ATCC 9184).The two extracts did not have any inhibitory effect on *Pseudomonas aeruginosa* (ATCC- 25619), Table 3.

The results of this study reveal the antibacterial activity of stem-bark of AO on the selected gram-negative bacteria. The methanolic extract seems to be more active against the microorganisms than the petroleum spirit extract and this could be ascribed to its ability to extract more active principles. Although the methanolic extract exhibited higher activity, but generally, the plant manifested significant therapeutic potentials. This is not surprising because the plant contains highly active compounds with antibacterial activities. There are tannins which are reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins (Westendarp, 2006; Trease and Evans, 2000).Steroids are also there, which are important drugs used as hypotensives, cardiac depressants, sedatives and anti-dysenteric agents (Abdul,1990).the glycosides which are used as laxative and carthatic drugs

were confirmed. Alkaloids that act as anti-malarial, anti-amoebic agents, astringents were present (Abdul, 1990).

Results obtained from this study could form a good basis for selection of AO for further phytochemical and pharmacological investigations. The antibacterial properties of AO can be exploited for potential development of new drugs against infectious diseases caused by the selected pathogens.

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